

AMENDMENTS TO THE CLAIMS:

1. *(Currently Amended)* A method of producing a heterologous peptide, polypeptide or protein in a lactic acid bacterium, the method comprising the steps of

(i) constructing a recombinant lactic acid bacterium comprising a nucleotide sequence coding for the heterologous peptide, polypeptide or protein and operably linked thereto, appropriate regulatory nucleotide sequences to control the expression of the coding sequence,

(ii) cultivating said recombinant bacterium under fed-batch or continuous cultivation conditions in a chemically defined medium, to express the gene, and

(iii) harvesting the recombinant bacterium or the peptide, polypeptide or protein.

2. *(Original)* A method according to claim 1 wherein the recombinant cell comprises a constitutive promoter operably linked to the coding sequence.

3. *(Currently Amended)* A method according to claim 1 wherein the recombinant cell comprises a regulatable promoter operably linked to the coding sequence.

4. *(Currently Amended)* A method according to claim 3 wherein the regulatable promoter is regulated by ~~a factor selected from the group consisting of pH, the growth temperature, the oxygen content, a temperature shift eliciting the expression of a heat shock gene, the composition of the growth medium including the ionic strength and the NaCl content, the presence/absence of an essential cell constituent or precursors herefor,~~ accumulation of a metabolite intracellularly or in the medium, the growth phase of the lactic acid bacterium and the growth rate of the lactic acid bacterium.

5. *(Currently Amended)* A method according to claim 3 or 4 wherein the regulatable promoter is derived from a lactic acid bacterium.

6. *(Currently Amended)* A method according to claim 5 wherein the regulatable promoter is the ~~pH-regulatable~~ P170 promoter disclosed in WO 98/10079 or a derivative thereof ~~which is pH-regulatable~~.

7. *(Original)* A method according to claim 1 or 2 wherein the promoter is introduced into the lactic acid bacterium on an autonomously replicating replicon.

8. *(Currently Amended)* A method according to claim ~~1 or~~ 2 wherein the promoter is a promoter not naturally associated with the nucleotide sequence coding for the heterologous peptide, polypeptide or protein.

9. *(Original)* A method according to claim 1 wherein the heterologous peptide, polypeptide or protein is selected from the group consisting of an enzyme and a pharmaceutically active compound.

10. *(Original)* A method according to claim 1 wherein the coding nucleotide sequence is operably linked to a nucleotide sequence coding for a signal peptide (SP).

11. *(Original)* A method according to claim 10 wherein the signal peptide is selected from the group consisting of the Usp45 signal peptide and the signal peptide having the sequence MKFNKKRVAIATFIALIFVSFFTISSQDAQAAERS (SEQ ID NO: 1).

Claim 12 (Cancelled)

13. *(Currently Amended)* A method according to claim ~~12~~ 1 wherein the concentration of glucose is kept at a pre-selected concentration of at least about 0.5 g/L by controlled feeding of glucose.

14. *(Original)* A method according to claim 13 wherein the control of feeding of glucose to the medium is linked to pH control.

15. *(Currently Amended)* A method according to claim ~~12~~ 1 wherein the chemically defined medium is supplemented with yeast extract.

16. *(Original)* A method according to claim 15 wherein the amount of yeast extract is in the range of 0.1-10 g/L.

17. *(Currently Amended)* A method according to ~~any of claims 1-4 and 9-11~~ claim 1 wherein the yield of heterologous peptide, polypeptide or protein is at least 5 mg/L.

18. *(Original)* A method according to claim 17 wherein the yield of heterologous peptide, polypeptide or protein is at least 100 mg/L.

19. *(Original)* A method according to claim 18 wherein the yield of heterologous peptide, polypeptide or protein is at least 200 mg/L.

Claims 12-23 (Cancelled)

24. *(Currently Amended)* A method according to claim ~~12~~ 1 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6

Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

^a From Neidhardt et al. J. Bacteriol. 119:736-747;

^b Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

^c Micronutrients: 0.003 μM (NH₄)₆(MoO₇)₂₄, 0.4 μM H₃BO₄, 0.03 μM CoCl₂, 0.01 μM CuSO₄, 0.08 μM MnCl₂ and 0.01 μM ZnSO₄.

25. *(Currently Amended)* A method according to claim 12 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6
Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a

FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

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^b Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate;

^c Micronutrients: 0.003 μM (NH₄)₆(MO₇)₂₄, 0.4 μM H₃BO₄, 0.03 μM CoCl₂, 0.01 μM CuSO₄, 0.08 μM MnCl₂ and 0.01 μM ZnSO₄;

wherein the components of said chemically defined medium are present in three-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

26. *(Currently Amended)* A method according to claim 12 1 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6

Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

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^c Micronutrients: 0.003 μM (NH₄)₆(MoO₇)₂₄, 0.4 μM H₃BO₄, 0.03 μM CoCl₂, 0.01 μM CuSO₄, 0.08 μM MnCl₂ and 0.01 μM ZnSO₄;

wherein the components of said chemically defined medium are present in five-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

27. *(Currently Amended)* A method according to claim 42 1 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3

L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6
Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

^a From Neidhardt et al. J. Bacteriol. 119:736-747;

^b Vitamins: 0.4 µM biotin, 10 µM pyridoxal-HCl, 2.3 µM folic acid, 2.6 µM riboflavin, 8 µM niacinamide, 3 µM thiamine-HCl and 2 µM pantothenate;

^c Micronutrients: 0.003 µM (NH₄)₆(MoO₇)₂₄, 0.4 µM H₃BO₄, 0.03 µM CoCl₂, 0.01 µM CuSO₄, 0.08 µM MnCl₂ and 0.01 µM ZnSO₄;

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L.

28. *(Currently Amended)* A method according to claim ~~12~~ 1 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5

L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6
Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

^a From Neidhardt et al. J. Bacteriol. 119:736-747;

^b Vitamins: 0.4 µM biotin, 10 µM pyridoxal-HCl, 2.3 µM folic acid, 2.6 µM riboflavin, 8 µM niacinamide, 3 µM thiamine-HCl and 2 µM pantothenate;

^c Micronutrients: 0.003 µM (NH₄)₆(MoO₇)₂₄, 0.4 µM H₃BO₄, 0.03 µM CoCl₂, 0.01 µM CuSO₄, 0.08 µM MnCl₂ and 0.01 µM ZnSO₄;

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L, and the components of said chemically defined medium are present in three-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.

29. *(Currently Amended)* A method according to claim 12 1 wherein the chemically defined medium is the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7

L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K ₂ SO ₄	0.28 ^a
KH ₂ PO ₄ /K ₂ HPO ₄	4/6
Na-acetate	15
CaCl ₂	0.0005 ^a
MgCl ₂	0.52 ^a
FeSO ₄	0.01 ^a
Vitamins ^b	+
Micronutrients ^{a,c}	+
Citric acid	0.1

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^b Vitamins: 0.4 µM biotin, 10 µM pyridoxal-HCl, 2.3 µM folic acid, 2.6 µM riboflavin, 8 µM niacinamide, 3 µM thiamine-HCl and 2 µM pantothenate;

^c Micronutrients: 0.003 µM (NH₄)₆(MO₇)₂₄, 0.4 µM H₃BO₄, 0.03 µM CoCl₂, 0.01 µM CuSO₄, 0.08 µM MnCl₂ and 0.01 µM ZnSO₄;

wherein glucose is additionally included in the chemically defined medium in an amount in the range of 1-100 g/L, and the components of said chemically defined medium are present in five-fold amounts of the enumerated concentrations, except the phosphates and sodium acetate, the respective amounts of which are kept at the enumerated concentrations.